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(54) Title: ANTI-INFLAMMATORY CYCLOOXYGENASE-2 SELECTIVE INHIBITORS

(57) Abstract: Disclosed is a novel anti-inflammatory pharmaceutical composition that exhibits potent and selective inhibition of the cyclooxygenase-2 (COX-2) enzyme. The formulation includes of a hops extract that exhibits COX-2 selectivity as defined by dividing the IC50 COX-2/ IC5o COX-1 concentrations that are determined by testing with the William Harvey Whole Blood Assay (WHMA), and falls in the range from about 0.011 to about 0.333. Such compositions may also optionally contain high levels of alpha acids and low levels of beta acids, some flavonoid or polyphenolic compounds, and from little to no essential oils. Such compositions are useful for treating conditions that manifest as inflammatory pain, or are impacted by the COX-2 enzyme. The recited compositions are particularly beneficial for treating osteoarthritis and rheumatoid arthritis, and can be used for chronic pain with reduced gastric side effects.

ANTI-INFLAMMATORY CYCLOOXYGENASE-2 SELECTIVE INHIBITORS

FIELD OF THE INVENTION

The present invention is drawn to anti-inflammatory compositions and methods of reducing inflammation in warm-blooded animals. More specifically, the present invention is drawn to hops extracts having specific compositional properties for reduction in inflammation.

BACKGROUND OF THE INVENTION

Research into the mechanism of inflammatory pain led to the discovery of the biochemical pathway associated with inflammation. One such example of chronic inflammatory pain is osteoarthritis. The elucidation of this pathway led to the association of the pro-inflammatory prostaglandins, produced by the cyclooxegenase enzyme, with pain and inflammation. The first generation of anti-inflammatory pain relievers was classified as non-steroidal anti-inflammatory drugs or NSAIDs. Common NSAIDs such as aspirin, ibuprofen, naproxen, and indomethacin inhibit the cyclooxygenase enzyme, and thereby reduce inflammation by lowering the production of prostaglandin E-2. Many new NSAIDs were developed over the last 30 years, most of which are available by prescription only.

Until the emergence of the discovery of a second form of the cyclooxygenase enzyme, now called COX-2, there had typically been no distinction made between the various NSAIDs in terms of the mechanism of action and their effect. Typically, only the magnitude of pain relief, or the potency for inhibiting the COX enzyme was considered important. However, side effects from the use of NSAIDs by patients who suffer from chronic inflammatory pain began to emerge. The principle side effect was gastrointestinal toxicity, and it manifested in the form of gastric erosion, or erosion of the mucosal protective lining of the stomach. By the early 90s, as the incidence of osteoarthritis and rheumatoid arthritis increased, this side effect became significant, leading to over 16,500 deaths per year in the United States alone. A review article

related to this by Wolf, M et al., Gastrointestinal Toxicity of Nonsteroidal Antiinflammatory Drugs, The New England Journal of Medicine, Vol. 340, No. 24, 1888-1899 (1999), is hereby incorporated by reference in its entirety. According to this article, 13 of every 1,000 patients with rheumatoid arthritis who take NSAIDs for one year have a serious gastrointestinal complication. According to data from the National Center for Health Statistics and the Arthritis, Rheumatism, and Aging Medical Information System, yearly deaths from NSAID toxicity (1997) in patients suffering from rheumatoid arthritis or osteoarthritis constitute the 15th leading cause of death in America. This figure is similar to mortality from AIDS (16,685) and only slightly less than deaths from Leukemia (20,197), but considerably greater than the number of deaths from multiple myeloma, asthma, cervical cancer, or Hodgkin's disease.

While most NSAIDs are more selective for the COX-1 form of the enzyme, they also inhibit the COX-2 form to varying degrees. Some NSAIDs, such as indomethacin, reduce both COX-1 and COX-2 to the same degree. Surprisingly, NSAIDs can also induce or up-regulate COX-2.

The potency of NSAIDs to cause gastric erosion and rapidly induce COX-2 can be illustrated by observing data from animal studies in which COX-2 was induced in the rat stomach within 1 hour of administration of aspirin or indomethacin. Both short term and long term administration of NSAIDs have produced gastric erosion as verified by endoscopy studies. Long term studies are defined as NSAID ingestion for at least 3 months, but usually are done over 3-6 months.

In the late 90s, a new class of prescription drugs emerged termed the COX-2 inhibitors. The first two compounds in this class approved by the U.S. FDA were Celecoxib and Rofecoxib. These drugs inhibited COX-2 with little or no effect on COX-1, and were sufficiently potent to produce equivalent pain relief to other NSAIDs. While these compounds were no more effective than the NSAID pain relievers, reduced gastrointestinal toxicity typically occurs. However, improvement in this area is still needed.

Since the emergence of COX-2 inhibitors on the market, continued research and development related to improvements in compositions and methods has occurred. In accordance with this, it would be desirable to find compounds that exhibit good

selective COX-2 inhibition, while providing the least amount of cardiovascular or gastrointestinal side effects.

SUMMARY OF THE INVENTION

It has been recognized that it would be advantageous to develop and produce compounds that exhibit good selective COX-2 inhibition, while providing the least amount of cardiovascular or gastrointestinal side effects. As such, an anti-inflammatory composition can comprise a hops extract including a pharmaceutically effective amount of alpha acid and from 0.5 wt% to 10 wt% of beta acid. In another embodiment, an anti-inflammatory composition can comprise a hops extract having a WHMA IC_{50} COX-2/ IC_{50} COX-1 ratio from about 0.011 to about 0.333. These embodiments can include the presence of essential oils in an amount less than 1 wt%.

Additionally, various methods of reducing inflammation and minimizing gastric erosion in a warm-blooded animal are provided. One such method can include steps of formulating a hops extract comprising a pharmaceutically effective amount of an alpha acid and from 0.5 wt% to 10 wt% of a beta acid; and administering the hops extract to a warm-blooded animal. Another method can include steps of formulating a hops extract having a WHMA IC_{50} COX-2 to COX-1 ratio from about 0.011 to about 0.333; and administering the hops extract to a warm-blooded animal.

Additional features and advantages of the invention will be apparent from the following detailed description which illustrates, by way of example, features of the invention.

DETAILED DESCRIPTION OF THE PREFERRD EMBODIMENT(S)

Before particular embodiments of the present invention are disclosed and described, it is to be understood that this invention is not limited to the particular process and materials disclosed herein as such may vary to some degree. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only and is not intended to be limiting, as the scope of the present invention will be defined only by the appended claims and equivalents thereof.

In describing and claiming the present invention, the following terminology will be used.

The singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a dye" includes reference to one or more of such materials.

As used herein, the term "IC_{50}" or "IC_{80}" shall mean the concentration of the compound or formulation that produces a 50% or 80% inhibition, respectively, of COX-1 or COX-2, as described in the William Harvey Modified Human Whole Blood/Cell Assay (WHMA). More specifically, the terminology for quantifying the potency of a cyclooxygenase-2 inhibitor is the Inhibitory Concentration (IC) that produces a reduction of prostaglandin E-2 by 50%, termed the IC_{50}. Another index is the Inhibitory Concentration that produces an 80% reduction. This is called the IC_{80}. For purposes of the present invention, the term IC_{80} refers to the concentration of the compound that produced an 80% reduction in the principle pro-inflammatory cytokine or prostaglandin (PGE-2). Alternatively, the concentration of the compound capable of producing an 80% inhibition of the COX-2 enzyme could also be referred to as the IC_{80}. Likewise, the term IC_{50} refers to the concentration of the compound that produces a 50% reduction in PGE-2, or a 50% reduction in the activity of the COX-2 enzyme.

As used herein, the concentration of the COX inhibitor shall be designated as either micrograms per milliliter (abbreviated as μ/ml) or micro molar (abbreviated as μM).

As used herein, the COX ratios are calculated as the IC_{50} COX-2/ IC_{50} COX-1, or IC_{80}COX-2/ IC_{80}COX-1, but COX-2 will always be divided by COX-1.

The term "dosage form" or the step of "administering" includes administration in a form that include oral dosage forms, suppository dosage forms, parenteral dosage forms, sublingual dosage forms, transdermal or transmucosal dosage forms, or the like.

As used herein, the term "oral dosage form" shall mean a pharmaceutical formulation designed to be administered orally, and can include one or more of various pharmaceutical carriers and excipients. Oral dosage forms can be in a tablet, a capsule, or a buccal administration form, for example. Oral dosage form will be absorbed in a human or animal oral cavity or gastrointestinal tract.

The term "sustained release" include a dosage form, such as an oral dosage form, that can be configured release an active ingredient over an extended period of time, or which is designed to provide for a longer residence time of the compound in the blood stream, thereby increasing the length of pain relief. Such sustained release compositions can include various polymers, fibers, resins, waxes, oils, or other pharmaceutical excipients used by those skilled in the art of medicinal chemistry to produce a prolonged release of the active constituents from the gastrointestinal tract, blood stream, etc.

With these definitions in mind, the present invention provides a composition that exhibits a selective inhibition of the COX-2 isoform of the cyclooxygenase enzyme while having at least a minimal effect on the COX-1 isoform. Minimal effect, for purposes of definition, shall mean at least 1% COX-1 inhibition activity. For example, a greater than 1% inhibition of COX-1 would correspond to a ratio of IC_{50} COX-2/ IC_{50} COX-1 of about 1:90 or about 0.011, in other words, it would take 90 times more of the compound to inhibit COX-1 by 50% than the amount to inhibit COX-2. In accordance with this, in one embodiment, an anti-inflammatory composition can comprise a hops extract having a WHMA IC_{50} COX-2/ IC_{50} COX-1 ratio from about 0.011 to about 0.333. In another related embodiment, an anti-inflammatory composition in accordance with embodiments of the present invention can comprise a hops extract including a pharmaceutically effective amount of alpha acid and from 0.5 wt% to 10 wt% of beta acid. These embodiments can include the presence of essential oils in an amount less than 1 wt%.

Additionally, various methods of reducing inflammation and minimizing gastric erosion in a warm-blooded animal are provided. One such method can include steps of formulating a hops extract comprising a pharmaceutically effective amount of an alpha acid and from 0.5 wt% to 10 wt% of a beta acid; and administering the hops extract to a warm-blooded animal. Another method can include steps of formulating a hops extract having a WHMA IC_{50} COX-2 to COX-1 ratio from about 0.011 to about 0.333; and administering the hops extract to a warm-blooded animal.

An additional aspect of the invention can include preparing a formulation that, upon administration, exhibits reduced gastrointestinal and cardiovascular side effects. Still further, an additional aspect includes the treatment of a disease impacted by the COX-2 enzyme, especially inflammation, or a disease that manifests in the up-

regulation or induction of COX-2. Some examples of such diseases include, but are not limited to; osteoarthritis, rheumatoid arthritis, dysmenorrea, and psoriasis. More generally, the compositions described herein may be used to treat any type of inflammation or pain associated with inflammation.

Compositions are also described that include primarily the alpha acids in hops, with little to no beta acids, and little to no essential oils. Furthermore, compositions that contain the various iso-alpha acids such as iso-humulone, iso-cohumulone, iso-adhumulone, trans-iso-humulone, cis-iso-humulone, trans-iso-cohumulone, cis-iso-cohumulone, cis-iso-adhumulone, trans-iso-adhumulone, dihydro-iso-humulone, and combinations thereof, may also be included.

Such compounds would result in a broader spectrum of therapeutic benefit, and be tunable over a wide range of COX-2/COX-1 ratios, providing effective pain relief with reduced side effects. Such compounds can also provide some minimal amount of COX-1 inhibition for cardiovascular benefit, without significant gastric erosion, while providing significant COX-2 inhibition for pain.

COX-2, or cyclooxygenase-2 inhibitors, inhibits cyclooxygenase and reduces prostaglandins without producing the degree of gastric erosion associated with NSAID drugs such as aspirin. A COX-2 inhibitor selectively inhibits the COX-2 form of the enzyme more than the COX-1 form. To be classified as a good COX-2 inhibitor, a compound should inhibit COX-2 at least three to five times more than COX-1. A good COX-2 inhibitor would be capable of producing a concentration level in the blood that would reduce pain by 80 to 90% by inhibiting COX-2, with little or no effect on the COX-1 form of the enzyme.

In-vitro testing or screening of COX-2 inhibitors can be conducted by measuring the inhibition of prostaglandin E-2, a pro-inflammatory prostaglandin, in human whole blood. This result in the calculation of the IC_{50} values, or the amount or concentration of the compound needed to inhibit COX-2 by 50%, or the IC_{80} value, the concentration of the compound necessary to reduce prostaglandin E-2 by 80%. This testing model measures the production of prostaglandin E2 (PGE2) by the COX-2 enzyme related pathways, when stimulated by LPS or some other inducer of the COX-2 enzyme. COX-1 activity can be measured by measuring the production of thromboxane (TxB2). Such assays are now considered to represent a more complete in-vitro picture of COX-2/COX-1 selectivity and potency.

In more detail, to determine the COX-2/COX-1 inhibitory activity, the William Harvey Modified Human Whole Blood /Cell Assay (WHMA) can be used, which is set forth in T. D. Warner et al., Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full *in vitro* analysis, Proc. Natl. Sci. USA 96:7563-68 (1999), which is hereby incorporated by reference in its entirety. The results from this assay can be used to calculate the IC_{50} and/or the IC_{80} WHMA COX-2/COX-1 ratio, which is simply the numerical ratio of the COX-2 IC_{50} (or IC_{80}) concentration divided by the COX-1 IC_{50} (or IC_{80}) concentration. In addition, the potency of the compound for reducing or inhibiting COX-2 can be thereby determined. This is done by measuring the inhibition of the two isoforms of the enzyme at different concentrations of the inhibitor, starting at very low concentrations, and increasing in a log fashion until at least an 80% inhibition is produced. This results in a log graph of the concentration versus inhibition curve, or a dose response curve.

An international group of scientists published a consensus review related to COX-2 screening assays in: Brooks et al; Interpreting the clinical significance of the differential inhibition of cyclooxygenase-1 and cyclooxygenase-2, Rheumatology 1999; 38: 779-788. In this consensus paper, the committee stated that the Human Whole Blood Assay developed by Patrignani et al (J Pharmacol Exp Ther 1994; 271: 1705-12) was the best assay available for assessing inhibition of COX-1 and COX-2, or evaluating new COX inhibitors. More recently, the William Harvey Modified Human Whole Blood Assay was developed as an extension of the original whole blood assay, and most of the NSAID drugs, as well as the newer COX-2 inhibitors have been screened using this method. The results from this assay are used to calculate the IC_{50} WHMA COX-2/COX-1 ratio, which is simply the numerical ratio of the COX-2 IC_{50} divided by the COX-1 IC_{50} ratio, obtained using the WHMA.

Human whole blood (8 concentrations, n=4) can be collected by venapuncture into heparin. For determining COX-1: Incubation of test compound(s) can be carried out for 1 hour, with addition of stimulus (A23187) for 30 minutes. For determining COX-2: Incubation of test compounds in A549 cells in human whole blood can be carried out for 1 hour, with addition of stimulus (A23187) for 30 minutes. Following this, TxB2 can be measured by RIA as an index of COX-1 activity, and PGE2 can be

measured by RIA as an index of COX-2 activity. The results can be expressed as % control, and COX-2/COX-1 ratio is calculated.

Returning to the problems associated with the relative incidence of GI side effects from NSAIDs, such side effects can be correlated to the relative COX-2 specificity of these anti-inflammatory agents. The higher the specificity for COX-2 over COX-1, the lower the incidence of GI upsets. Accordingly, cyclooxygenase inhibiting agents with increased COX-2 specificity may provide improved anti-inflammatory compositions having less incidences of gastrointestinal distress or side effects. It is becoming increasingly apparent that the gastric damage that can be caused by NSAIDs is not just related to their effect on COX-1. Dual suppression of both COX-1 and COX-2 can cause damage to occur (Wallace, JL et al., NSAID-Induced Gastric Damage in the rat: Requirement for Inhibition of Both Cyclooxygenase-1 and Cyclooxygenase-2. Gastroenterology, 2000; 119:706-14). Furthermore, selective inhibition of COX-1, which greatly reduced prostaglandin synthesis, did not produce gastric damage in the same study. On the other hand, selective inhibition of COX-2 did not appear to have any effect on gastric prostaglandin synthesis, and did not produce gastric damage. Interestingly, when both COX-1 and COX-2 were inhibited, gastric damage was consistently observed. This, and other research, is providing a clearer picture of the relationship between COX-1, COX-2, and gastric erosion. It now appears that combined inhibition of COX-1 and COX-2 contribute to the side effects, but more highly selective inhibition of either COX-1 or COX-2 alone, is not responsible.

However, it has been recognized that too much selectivity for COX-2 over COX-1 may not be desirable for other reasons. Certain side effects may result from COX inhibitors that are extremely selective for COX-2. For example, the cardiovascular benefit of aspirin, a predominantly COX-1 non-steroidal anti-inflammatory drug (NSAID), is thought to be due to its activity as an anti-platelet aggregating drug. COX-2 inhibition does not result in anti-platelet aggregation. Current pharmaceutical COX-2 inhibitors, such as Celecoxib or Rofecoxib, are highly specific COX-2 inhibitors, and would not be expected to have any COX-1 inhibitory activity at the doses used to reduce pain and inhibit COX-2 activity. Thus, the cardiac-related side effects that have been noted with the use of some COX-2 specific

inhibitors may be related to the lack of any COX-1 inhibition while significantly inhibiting COX-2.

Furthermore, it has also been recognized that an additional problem associated with highly specific COX-2 inhibitors is the increase in gastric erosion produced by concurrent administration with other non-steroidal anti-inflammatory drugs (NSAIDS). For example, if a patient is taking a highly selective COX-2 inhibitor and also takes aspirin for cardiovascular benefit, the aspirin will cause even worse damage to the gastric mucosa. Without being bound by any particular theory, the reason for this may be because some of the prostaglandins that are inhibited by cyclooxygenase inhibitors, such as prostaglandin E-2 (PGE2), are protective of the gastric mucosa, and actually contribute to healing of ulceration. Low dose aspirin produces small erosions in the stomach, and at the site of these ulcerations, the COX-2 enzyme becomes up-regulated. When COX-2 is blocked by selective COX-2 inhibitors, the protection afforded by the beneficial prostaglandins may be eliminated. Concomitant administration of selective COX-2 inhibitors with aspirin is therefore contraindicated. This phenomenon is an indication of the problems associated with the dual inhibition of both COX-1 and COX-2. Thus gastric erosion may even be worse with a single compound that exhibits significant inhibition of both COX-1 and COX-2, or by combining a COX-2 selective compound with a non-selective COX inhibitor that also inhibits COX-1 to a large degree.

Highly selective single entity COX-2 inhibitors such as Rofecoxib and Celecoxib, while important new drugs for the treatment of pain associated with osteoarthritis and other maladies, have some serious potential side effects. These side effects can be divided into two major groups; 1) cardiovascular, and 2) worsening of gastric erosion when taken with aspirin or other NSAIDS. Both of these side effects may be related to an unbalanced total inhibition of the COX enzyme, and therefor, virtually complete blocking of prostaglandin production. Because prostaglandins have both positive and negative functions in the body, their total inhibition is a double-edged sword. Furthermore, there is a significant overlap in the patient populations that take both aspirin for cardiovascular benefit, and a selective COX-2 inhibitor for pain. Most of these subjects primarily consist of the elderly population. There is a significant need for anti-inflammatory pain relief without the negative side effects of the NSAIDs or the selective COX-2 inhibitors. Such a composition would provide

pain relief while also inhibiting platelet aggregation, and providing protection for the gastric mucosa through some gastroprotective or cytoprotective mechanism. These second generation COX-2 inhibitors would be selective enough to inhibit COX-2 over COX-1, but not so selective that they would result in the additional side effects mentioned above. These compounds may exhibit protective activity by virtue of the existence of some other beneficial properties.

In accordance with these recognitions, it has been determined in accordance with embodiments of the present invention that a key to overall risk reward benefits would be to have just the right amount of COX-1 inhibition along with predominantly COX-2 inhibition. Such an IC_{50} COX-2/ IC_{50} COX-1 can be from about 1:90 to about 1:3, or numerically from about 0.011 to about 0.333. In another embodiment, the IC_{50} COX-2/ IC_{50} COX-1 can be from about 1:50 to about 1:20, or numerically from about 0.02 to about 0.05. In another embodiment, the ratio of IC_{50} COX-2/ IC_{50} COX-1 can be at least 1:20, or numerically at least 0.05. Further, in one specific embodiment, a compound that is tested using the WHMA protocol might have an IC_{50} for COX-2 of 1 μg/ml and an IC_{50} for COX-1 of 20 μg/ml, resulting in an IC_{50} COX-2/ IC_{50} COX-1 ratio of about 1:20 or 0.05.

In the search for new anti-inflammatory compounds, many potential candidates have come from the plant kingdom. These botanicals are usually extracted and tested in-vitro for COX inhibition using various cell lines and methods. Usually these methods involve screening the compounds for COX-2 and COX-1 inhibition by measuring the inhibition of prostaglandin E-2 for COX-2 inhibition, and TxB2 for COX-1 inhibition. Selectivity can then be determined by calculating the COX-2/COX-1 ratio, as calculated herein. Ratios can also be calculated by a COX-1/COX-2 ratio, and though different numbers will be obtained, the relative values could be used to identify ratios effectively.

One such candidate is a special extract of Humulus lupulus L., or the plant commonly known as hops. Hops is derived from the cone flowers of the hops plant, and has been used in the production of beer for 100s of years. Hops itself may exhibit some metabolic and endocrine effects. There are at least six flavonoids or polyphenolic compounds that can be isolated from hops, and which can be present in accordance with embodiments of the present invention. Some of these flavonoids have antiproliferative, estrogenic, and cytotoxic effects. The phytoestrogens in hops

have also been shown to inhibit growth of human breast cancer cells. The unique flavonoid compounds isolated from hops (prenylated flavanoids) therefore may have potential as cancer chemopreventative agents by affecting the metabolism of carcinogens. The flavones contained in hops include xanthohumol, isoxanthohumol, desmethylxanthohumol, 8-prenylnaringenin, 6-prenylnaringenin, and various other flavonoids. Hops can also exhibit antimicrobial and anti-fungal properties.

The primary constituents in hops include alpha acids and beta acids. The alpha acids have been identified as humulone, cohumulone, adhumulone, dihydrohumulone, and dihydroadhumulone. These alpha acids also exist as dihydro-alpha acids and as various isomers. The iso-alpha acids are iso-humulone, iso-cohumulone, iso-adhumulone, trans-iso-humulone, cis-iso-humulone, trans-iso-cohumulone, cis-iso-cohumulone, cis-iso-adhumulone, trans-iso-adhumulone, dihydro-iso-humulone, and dihydro-iso-adhumulone. The beta acids are lupulone, colupulone, adlupulone, prelupulone, and postlupulone. There are no isomers of the beta acids. Hops also contains various essential oils such as myrcene, caryophyllene, humulene, undecane-2-on, and 2-methyl-but-3-en-ol. These oils can be classified primarily as terpenes and sesqueterpenes. At least 50% of the essential oils include of the terpene, myrcene.

Topical application of humulone, one of the alpha acids isolated from hops, inhibited arachidonic acid-induced inflammatory ear edema in mice (Yasukawa, K et al, Oncology 1995, Mar; 52 (2): 156-158), and also inhibited skin tumor formation following initiation with a chemical challenge. Pure humulone, has also been shown to suppress cyclooxygenase-2 induction at the level of gene transcription (Yamamoto K, et al, FEBS Lett 2000 Jan 14, 465(2-3: 103-106). In this same study, humulone inhibited the catalytic activity of COX-2 in osteoblast (bone) MC3T3-E1 cells with an IC_{50} of 1.6 μM. Furthermore, humulone suppressed the TNF-alpha-dependent cyclooxygenase-2 induction in the same cell line. The direct inhibition of the COX-2 enzyme by humulone required a greater concentration than the concentration necessary to inhibit the gene transcription, or the suppression of COX-2 expression. Humulone appeared to be more effective at a lower concentration in preventing the transcription or activation of COX-2 by suppressing the gene transcription, than by direct inhibition of the COX-2 enzymes catalytic activity. The IC_{50} for suppression of COX-2 transcription was 30 ηM (10^{-9}) whereas the IC_{50} for direct inhibition of

catalytic activity was 1.6 μM (10^{-6}), or two orders of magnitude lower. Only pure humulone was used in this study.

Special extracts of hops cone flowers can preferably be prepared employing supercritical carbon dioxide. Supercritical CO_2 extraction can result in extracts of hops that contain a very high percentage of alpha acids, a very low percentage of beta acids, and essentially no essential oils. This invention, however, is not limited to the extraction technique. Preferred amounts of alpha acids in the instant invention can be from 75 wt% to 99.5 wt%. The alpha acids may be humulone, cohumulone, adhumulone, dihydrohumulone, or tetra-hydro-alpha acids such as tetra-hydrohumulone. The beta acids can be from 0.5 wt% to 10 wt%, and in another embodiment, from about 3 wt% to 5 wt%. Optionally, the composition may be substantially devoid of essential oils such as myrcene or other terpenes or sesqueterpenes. By substantially devoid, what is meant is that essential oils are not present except for in trace or insignificant amounts. In another embodiment, the essential oils can be present in less than a 1 wt% amount. Additionally, the composition may also contain iso-alpha acids to varying degrees. Usually the level of iso-alpha acids will be not more than 10 wt%, such as from 0.5 wt% to 10 wt%. The iso-alpha acids may be iso-humulone, iso-cohumulone, iso-adhumulone, di-hydro-iso-humulone, di-hydro-iso-adhumulone or combinations thereof. The iso-alpha acids may be useful for tuning the selectivity for COX-2 in the formulation, by boosting the COX-1 component, and changing the COX-2/COX-1 ratio to be less selective for COX-2. The reason for these types of formulas may be to address cardiovascular issues by contributing some anti-platelet aggregation activity from the COX-1 inhibition via thromboxane. In this embodiment, the alpha acids that are non iso-alpha acids can be present in amount greater than about 80 wt%.

As previously stated, the IC_{50} ratio of COX-2 to COX-1 or the IC_{50} COX-2/ IC_{50} COX-1 can be in the range of about 0.011 to about 0.333, or from about 1:90 to about 1:3. The percentage of alpha acids can be form 60 wt% to 99.5 wt%, but is not limited to this range if the IC_{50} COX-2/ IC_{50} COX-1 ratios fall within the specified ranges of 0.011 to 0.333. In another embodiment, the range can be from 0.13 to 0.05, and in still further detail, can be from 0.02 to 0.05. As mentioned, the iso-alpha acids may be from 0.5 wt% to 10 wt%. Independent of this, the beta acids can range can be from 0.5 wt% to 10 wt%. Additionally, various polyphenols or flavonoids may be

present such as xanthohumol, isoxanthohumol, 8-prenylnaringenin, 6-prenylnaringenin in varying amounts.

In embodiments related to the WHMA ratios, the compositions in accordance with the present invention are not limited to the amount of alpha acids and beta acids, or the method of processing or extraction. For example, a hops extract that exhibits a WHMA IC_{50} COX-2 over IC_{50} COX-1 ratio of 0.013 (1:75) would be part of this invention regardless of the method of processing or the amount of alpha acids, beta acids, or essential oils. In one embodiment, as stated, powders of hops can be made which exhibit IC_{50} COX-2/COX-1 ratios of about 1:20, or numerically 0.05, which provide good pain relief from chronic osteoarthritis or rheumatoid arthritis. Such compositions can also be useful for treating dysmenorrhea or menstrual pain, psoriasis, and other diseases impacted by COX-2.

EXAMPLES

The following examples illustrate the embodiments of the invention that are presently best known. However, it is to be understood that the following are only exemplary or illustrative of the application of the principles of the present invention. Numerous modifications and alternative compositions, methods, and systems may be devised by those skilled in the art without departing from the spirit and scope of the present invention. The appended claims are intended to cover such modifications and arrangements. Thus, while the present invention has been described above with particularity, the following Examples provide further detail in connection with what are presently deemed to be the most practical and preferred embodiments of the invention.

Example 1: COX-2 inhibition activity of hops

A supercritical carbon dioxide extract of hops was produced that yielded 91 wt% alpha acids, of which the principle alpha acid was humulone as verified by HPLC. The amount of beta acids in this extract was verified to be 3.2 wt% and the amount of iso-alpha acids was about 3 wt%. This extract was virtually devoid of the essential oils normally found in a typical hops powder or extract.

Hops extract constituents by HPLC:

Alpha acids	88 wt%
Beta acids	3.2 wt%
Iso-alpha acids	3 wt%
Total alpha acids	91 wt% (alpha and iso-alpha)

This extract was dissolved in DMSO and tested according to the protocol described above. The effects of test agents on COX-1 and COX-2 activity are detailed in Tables 1-3. Results in Tables 1-3 are expressed as % control and shown as mean ± s.e.m. (n=4) from which IC_{50} values were calculated (Table 3).

Table 1

COX-1 activity in human whole blood (TxB_2, % control)								
	Concentration (log M)							
	-10	-9	-8	-7	-6	-5	-4	-3
Hops extract	100±6	103±6	100±5	94±4	91±5	80±10	60±15	18±2

Table 2

COX-2 activity in A549 cells in human whole blood (PGE_2, % control)								
	Concentration (log M)							
	-10	-9	-8	-7	-6	-5	-4	-3
Hops extract	97±6	95±6	87±8	61±8	54±10	42±10	16±3	6±2
Ibuprofen		85±16	82±10	80±9	80±5	60±4	20±5	4

By way of explanation, log M refers to the log of the molar concentration of the compound being tested. For example, 10^{-9} would be the log of the nanomolar

concentration, and 10^{-6} would be the log of the micromolar concentration, and so forth. The values for hops extract and ibuprofen are based on a percentage of the control (which is the DMSO solvent without anything added thereto).

As can be seen from tables 1 and 2, a rather large amount of the hops extract is necessary to reduce COX-1 by 50% (the IC_{50} is 10^{-3} to 10^{-4}), whereas, for COX-2, the concentration needed for 50% reduction is 10^{-6} (IC_{50} was 1.4 x 10^{-6} μM). It takes about 100 times as much of this 91 wt% alpha acid hops extract to reduce COX-1 by 50% as the concentration needed to reduce COX-2 activity by 50%. Ibuprofen is included for comparison. The hops extract was more potent and selective than ibuprofen for inhibition of COX-2.

Table 3

Potencies of test agents on human COX-1 and COX-2 (WHMA)			
	IC_{50} (μM)		IC_{50} ratio (COX-2/1)
	COX-1	COX-2	
Aspirin	1.7	7.5	4.4
Ibuprofen	7.6	20	2.6
Naproxen	9.3	35	3.8
Hops extract	110	1.4	0.0127
Celexocib	2.2	0.34	0.3
Rofecoxib	63	0.31	0.0049
Indomethacin	0.0031	0.021	7

Table 3 is a comparison of the 91 wt% alpha acid containing hops extract that was derived by supercritical carbon dioxide, with other known COX inhibitors, including the prescription COX-2 selective inhibitors Rofecoxib and Celexocib, as well as the non selective COX inhibitors aspirin, ibuprofen, naproxen and

indomethacin. As can be seen from the above data, hops extract is a selective and potent COX-2 inhibitor when tested according to the WHMA protocol. The IC_{50} COX-2 concentration of this hops extract was about 1.4 μM, a concentration that would be within a range of pharmacological action of most non-steriodal anti-inflammatory drugs, whereas the IC_{50} COX-1 concentration was about 110 μM. The ratio was therefor 0.0127 or 1:90.

Table 4

Potencies of test agents on human COX-1 and A549 COX-2 (WHMA)						
	IC_{50} (μM)		IC_{80} (μM)		ratios (COX-2/1)	
	COX-1	COX-2	COX-1	COX-2	IC_{50}	IC_{80}
Hops extract	110	1.4	>1000	85	0.01	<0.09
Indomethacin	0.0069	0.055	0.027	0.21	8	8

Table 4 includes the IC_{80} data for the special hops extract compared to indomethacin, a potent non-selective NSAID that lowers COX-1 and COX-2 at a very low concentration. As can be seen from table 4 above, the IC_{80} for COX-2 was about 85 μM, and the IC_{80} COX-2/1 ratio was about 0.09 μM, which is a ratio of about 1:11. While not as potent as indomethacin, the selectivity for COX-2 is much greater.

While the resin used in the above experiment resulted in significant inhibition of the COX-2 enzyme with very little effect on the COX-1 form, such a resin is difficult to use in a pharmaceutical dosage form without converting to a powder. When converted to a powder, various excipients can be used as carriers which tend to dilute the potency of the resin, thereby reducing the IC_{50} or IC_{80} by about 50%, and also enabling various mixtures of alpha acids and iso-alpha acids to be employed.

Example 2: COX-2 inhibition of a hops resin converted to powder

A hops resin is converted to powder using maltodextrin and calcium silicate in a jacketed high intensity mixer. The resulting powder was analyzed by HPLC and found to yield the following principle constituents:

Hops powder constituents by HPLC:

Alpha acids	20 wt%
Iso-alpha acids	9.4 wt%
Beta acids	8 wt wt%

This powder was tested for COX-2 and COX-1 inhibition in a cell line in whole blood by inducing COX-2 with LPS (lipopolysaccharide) and measuring PGE-2 for COX-2 activity. COX-1 activity was assessed by measuring TxB2 (thromboxane B2). IC_{50} results for COX-2 and COX-1 were as follows:

IC_{50} COX-2	IC_{50} COX-1	IC_{50} COX-2/ IC_{50} COX-1
1 μg/ml	30 μg/ml	0.033 (1:30)

Example 3: Gastric erosion of hops formulations vs. non-selective COX inhibitor

This example is to demonstrate the reduction in gastric erosion of a hops formulation versus a traditional non-selective COX inhibitor NSAID such as aspirin. An oral formulation of a tablet containing 750 mg of a hops extract powder including of 30 wt% alpha acids (225 mg. alpha acids) is administered to 40 subjects in a single blind, parallel-group, multiple dose study. The patients are randomly assigned to treatment with either the hops alone or 1,000 mg. aspirin per day for 4 days. Assessments are made based on endoscopic evaluations of gastroduodenal irritancy performed 4 hours after the first dose and 3 hours after the final administration on the fourth study day. To assure that all study subjects had normal healthy gastroduodenal mucosa at baseline, an endoscopic evaluation is also performed before subjects are randomized.

Endoscopy is used to assess the extent and severity of gastric and duodenal damage. During an endoscopic examination of the stomach and duodenum, the number and location of submucosal hemorrhages, erosions, and ulcerations, are determined by the endoscopist. Based on the findings, the hemorrhagic damage is graded on a scale of 0-4 and the erosive damage is graded on a separate 0-4 scale. The stomach and duodenum are graded separately. Under the test conditions described above, endoscopic evaluation is expected to reveal virtually no gastric erosion form

the hops formulation, while the aspirin formulation exhibited significant gastric submucosal hemorrhages and overall gastric erosions. The difference between the two groups is expected to be statistically significant. The reduction in gastric erosion of the hops formulation in comparison to aspirin is believed to be related to its selectivity for COX-2 over COX-1.

While the present invention is described above in connection with the preferred or illustrative embodiments, those embodiments are not intended to be exhaustive or limiting of the invention, but rather, the invention is intended to cover any alternatives, modifications, or equivalents that may be included within its scope as defined by the appended claims.

CLAIMS

What Is Claimed Is:

1. An anti-inflammatory composition, comprising a hops extract including a pharmaceutically effective amount of alpha acid, from 0.5 wt% to 10 wt% of beta acid, and less than 1 wt% of essential oils.

2. The composition of claim 1, wherein the alpha acid and beta acid are derived from hops cone flowers.

3. The composition of claim 1, wherein the alpha acid is selected from the group consisting of humulone, cohumulone, adhumulone, dihydrohumulone, dihydroadhumulone, and mixtures thereof.

4. The composition of claim 1, further comprising an iso-alpha acid selected from the group consisting of iso-humulone, iso-cohumulone, iso-adhumulone, dihydro-iso-humulone, dihydro-iso-adhumulone, and combinations thereof.

5. The composition of claim 4, wherein the iso-alpha acid comprises a member selected from the group consisting of trans-iso-humulone, cis-iso-humulone, trans-iso-cohumulone, cis-iso-cohumulone, cis-iso-adhumulone, trans-iso-adhumulone, and combinations thereof.

6. The composition of claim 1, wherein the alpha acid is present at from 75 wt% to 99.5 wt%.

7. The composition of claim 1, wherein the hops extract is substantially devoid of essential oils.

8. The composition of claim 1, further comprising a flavonoid or polyphenolic compound.

9. The composition of claim 1, wherein the hops extract comprises greater than 80 wt% alpha acid and not more than 10 wt% iso-alpha acid.

10. The composition of claim 1, further comprising a pharmaceutical carrier, and wherein the dosage form is for alimentary delivery in the form of a tablet, capsule, or suppository.

11. The composition of claim 11 in a sustained-release formulation.

12. The composition of claim 1, wherein the hops extract has a WHMA IC_{50} COX-2/ IC_{50} COX-1 ratio from about 0.011 to about 0.333.

13. The composition of claim 1, wherein the hops extract has a WHMA IC_{50} COX-2/ IC_{50} COX-1 ratio from about 0.02 to about 0.05.

14. An anti-inflammatory composition, comprising a hops extract having a WHMA IC_{50} COX-2/ IC_{50} COX-1 ratio from about 0.011 to about 0.333, wherein the hops extract includes less than 1 wt% of essential oils.

15. The composition of claim 14, wherein the hops extract comprises alpha acid and beta acid derived from hops cone flowers.

16. The composition of claim 14, wherein the hops extract is substantially devoid of beta acid.

17. The composition of claim 14, wherein the alpha acid is selected from the group consisting of humulone, cohumulone, adhumulone, dihydrohumulone, tetra hydro-alpha-acids, and mixtures thereof.

18. The composition of claim 14, further comprising an iso-alpha acid selected from the group consisting of iso-humulone, iso-cohumulone, iso-adhumulone, dihydro-iso-humulone and combinations thereof.

19. The composition of claim 18, wherein the iso-alpha acid comprises a member selected from the group consisting of trans-iso-humulone, cis-iso-humulone, trans-iso-cohumulone, cis-iso-cohumulone, cis-iso-adhumulone, trans-iso-adhumulone, and combinations thereof.

20. The composition of claim 14, wherein the hops extract includes alpha acid in an amount ranging from about 60 wt% to 99.5 wt%.

21. The composition of claim 14, wherein the hops extract includes beta acid in an amount ranging from about 0.5 wt% to 10 wt%.

22. The composition of claim 14, wherein the hops extract is substantially devoid of essential oils.

23. The composition of claim 14, wherein the hops extract further comprises a flavonoid or polyphenolic compound.

24. The composition of claim 14, wherein the hops extract comprises greater than 80 wt% alpha acid and not more than 10 wt% iso-alpha acid.

25. The composition of claim 14, comprising a pharmaceutical carrier, and wherein the dosage form is for alimentary delivery in the form of a tablet, capsule, or suppository.

26. The composition of claim 25 in a sustained-release formulation.

27. The composition of claim 14, wherein the hops extract comprises a pharmaceutically effective amount of alpha acid and from 0.5 wt% to 10 wt% of beta acid.

28. The composition of claim 14, wherein the hops extract has a WHMA IC_{50} COX-2/ IC_{50} COX-1 ratio range from about 0.02 to 0.05.

29. A method of reducing inflammation and minimizing gastric erosion in a warm-blooded animal, comprising:

formulating a hops extract comprising a pharmaceutically effective amount of an alpha acid and from 0.5 wt% to 10 wt% of a beta acid; and

administering the hops extract to a warm-blooded animal experiencing symptoms of inflammation.

30. The method of claim 29, wherein the step of administering is by oral delivery.

31. The method of claim 29, wherein the step of administering is by transmucosal delivery.

32. The method of claim 29, wherein the step of administering is by parenteral delivery.

33. The method of claim 29, wherein the hops extract has a WHMA IC_{50} COX-2/ IC_{50} COX-1 ratio from about 0.011 to about 0.333.

34. The method of claim 33, wherein the hops extract has a WHMA IC_{50} COX-2/ IC_{50} COX-1 ratio range from about 0.013 to 0.05.

35. A method of reducing inflammation and minimizing gastric erosion in a warm-blooded animal, comprising:

formulating a hops extract having a WHMA IC_{50} COX-2 to COX-1 ratio from about 0.011 to about 0.333; and

administering the hops extract to a warm-blooded animal experiencing symptoms of inflammation.

36. The method of claim 35, wherein the step of administering is by oral delivery.

37. The method of claim 35, wherein the step of administering is by transmucosal delivery.

38. The method of claim 35, wherein the step of administering is by parenteral delivery.

39. The method of claim 35, wherein the hops extract comprises a pharmaceutically effective amount of an alpha acid and from 0.5 wt% to 10 wt% of a beta acid.